

Figure 1.  
Cloning vectors for the expression of UDP and PNP enzymes

# Plasmid pUC18: 5' sequence of *lacZ* gene

RBS  
AGGAAAACAGCT ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG *SphI* *HindIII*  
thr met ile thr asn ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu

# plasmid pGM678 and pGM707: sequence of *lacZ*-*deoD* fused genes

RBS  
AGGAAAACAGCT ATG ACC ATG ATT ACG AAT TCT TCC ATG GCT ACC CCA.....TGG GCG TAA AGAGTAAGTCGACCTGC..... *SalI*  
thr met ile thr asn ser ser met ala thr pro.....trp ala stop

# plasmid pGM679 and pGM708: sequence of *lacZ*-*udp* fused genes

RBS  
AGGAAAACAGCT ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCA TCC ATG TCC .....CTG CTG TAA TTCTCTTGTGCAATG..... *SalI*  
thr met ile thr asn ser ser val pro ser met ser.....leu leu stop

# plasmid pGM712 e pGM716: 5' and 3' sequence of *deoD* gene

*SalI*/*NheI* RBS *EcoRI*  
GTGCTAGCAGGAGGAATTC ATG GCT ACC CCA..... TGG GCG TAA AGAGTAAGTCGACCTGCAGGCATGCAA *SphI*  
met ala thr pro..... trp ala stop

Figure 2. 5' and 3' sequences of *udp* e *deoD* genes cloned in plasmid pUC18. Restriction sites of different constructs are underlined; the ribosome binding site (RBS) is reported in bold. The bases of nucleotide sequence of *udp* and *deoD* genes and the amino acid residues of PNP and Udp proteins are reported in *italics*.

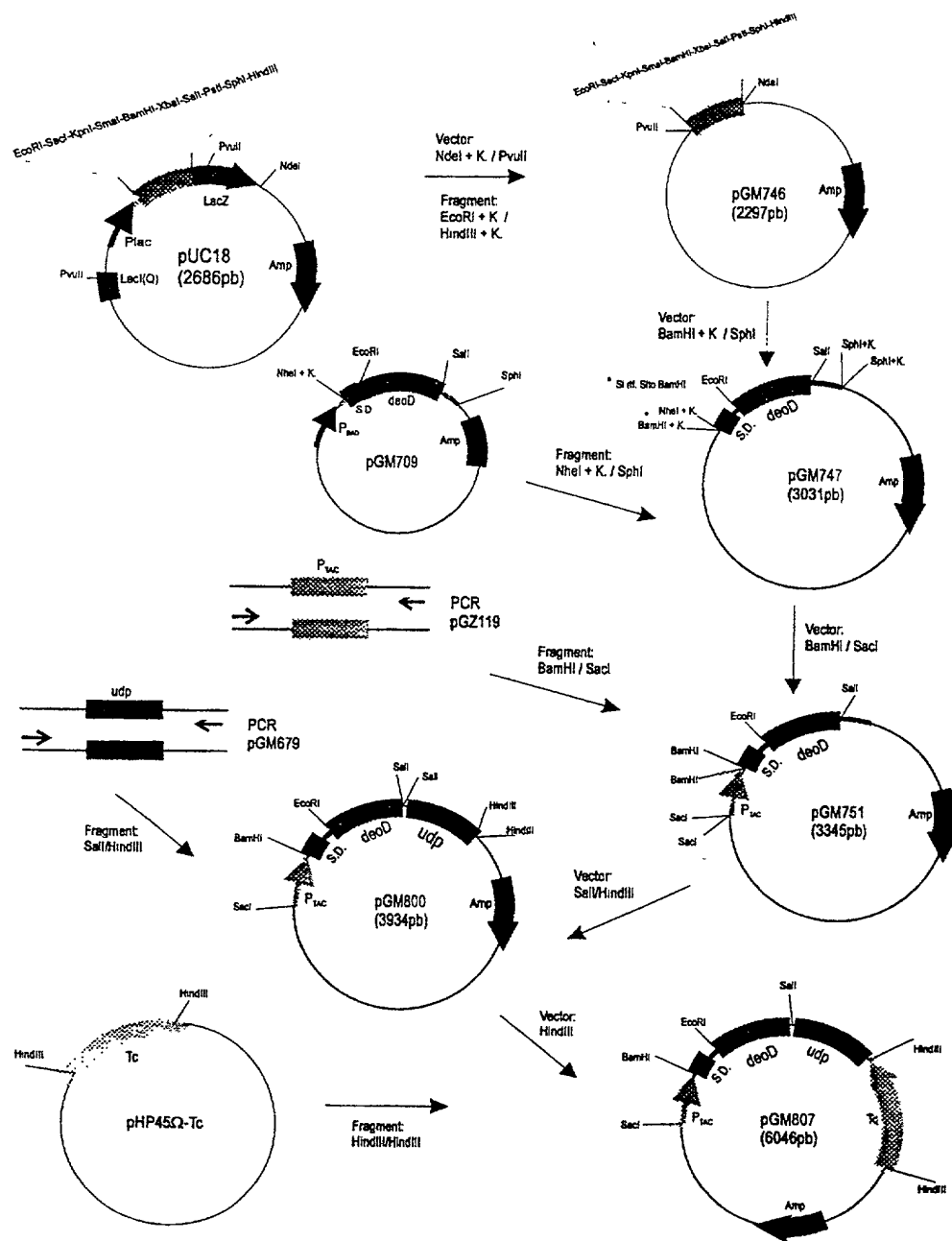


Figure 3.  
Costruction of cloning vectors for the expression of UdP and PNP enzymes

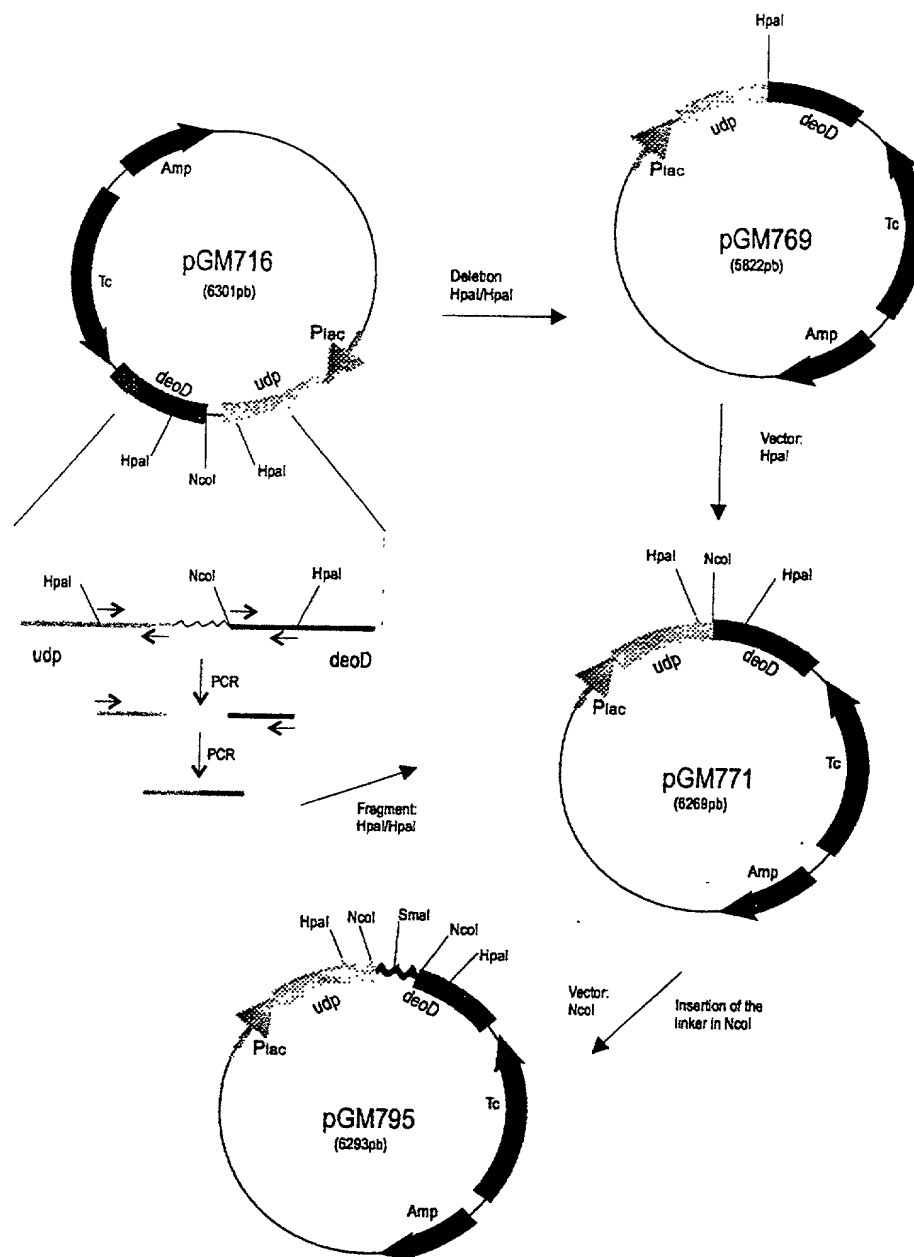


Figure 4.  
Construction of cloning vectors for the expression of UdP-(L)-PNP enzymes.

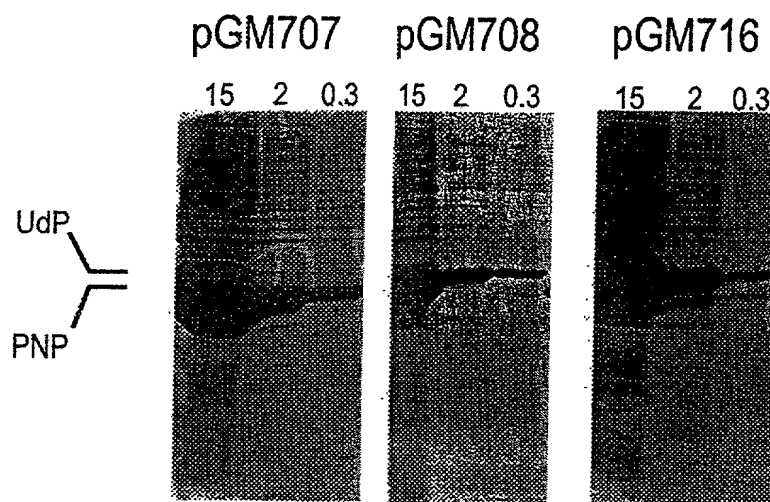


Figure 5.  
Expression of PNP and UdP in recombinant *E. Coli* strains. Gel electrophoresis (SDS-PAGE) of total protein extracts from strains MG1655/pGM707, MG1655/pGM708 and MG1655/pGM716 grown over night in LD medium supplemented with 12.5 mg/liter of tetracycline. Lanes 15, 2 and 0.3 correspond to protein extracted from 15, 2 and 0.3 ml of bacterial culture.